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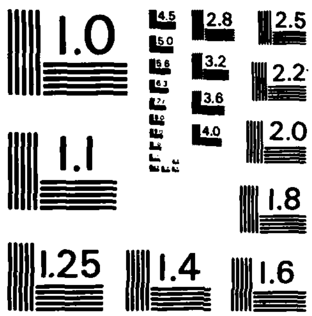

MICROCOPY RESOLUTION TEST CHART

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~~Studies on the Mechanism of Suppression of the Immune Response by Synthetic, Non-toxic adjuvants~~.

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Non-toxic synthetic adjuvants are under prime consideration for use in increasing the immune response of human beings. Three of microbial origin are the muramyl di-peptides (N-acetyl-muramyl-L-alanyl-D-isoglutamine and analogs, termed MDP), polyadenylic acid-polyuridylic acid complexes termed poly A·poly U) and the recently isolated monophosphoryl lipid A (termed MPL). While each of these has been demonstrated in animals to be active in increasing the immune response when given with the antigen, each also has been found to suppress this response when given one to several days before antigen. The enhancing actions of MDP and poly A·poly U have been well characterized. However, characterization of the suppressive phenomenon has been minimal, but is important to gain a responsible understanding of how these adjuvants regulate the immune response non-specifically. Accordingly, the experiments proposed during the tenure of this contract were undertaken to further our knowledge of how each of these adjuvants activate the suppressive arm of the immune response. The data are submitted in the form of four preprints and one reprint and

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permit the following main conclusions:

POLY A · POLY U

1. Poly A·poly inhibited antibody forming cells non-specifically when given 1-4 days before antigen, whereas poly I·poly C inhibited when given 1-6 days before antigen.
2. This suppression could be expressed in in vitro experiments by addition of surprisingly either T cell rich, B cell rich or adherent cell populations to their syngeneic normal cell counterparts, suggesting an unidentified cell may be contaminating each.
3. To determine whether an NK cell was contaminating the above 3 populations and was responsible for suppression, NK activity was removed with anti-asialo GM1 antibody without affecting the magnitude of the suppression.
4. Suppressive activity for both humoral and cell mediated immunity (MLR) was found and characterized in the serum of mice injected with poly A·poly U after 90 minutes.
5. Poly A·poly U increased non-specific resistance to Streptococcus pneumoniae and Pseudomonas aeruginosa when given 1-2 days before challenge with these microorganisms, despite the presence of antibody suppressing activity in the spleen at this time.

MURAMYL DI-PEPTIDES

1. A single injection of MDP either ip or iv, 1-2 days before antigen inhibited antibody forming cells by approximately 50%. This suppression lasted from 4-14 days with much individual variation.
2. Using derivatives of MDP it was shown that the muramyl grouping was not necessary for suppressive activity. The addition of an n-butyl ester grouping to the terminal carboxyl of the glutamine moiety of MDP did not increase the capacity to induce suppression.
3. Suppression could be transferred to syngeneic recipient mice with both adherent and non-adherent spleen cells. T cells were found to be the effector cell in the latter population.
4. Unlike poly A·poly U, MDP did not induce suppressive activity in the serum 90 minutes after injection.
5. Interleukin I activity was depressed 24 and 48 hr after MDP injection, while Il-2 activity became depressed later at 72 hr.
6. It was hypothesized that MDP initiates suppression in the macrophage population in the form of decreased Il-1 production, which in turn depressed Il-2 levels. The net result was a decrease in numbers of antibody forming cells.

MONOPHOSPHORYL LIPID A

1. A non-toxic monophosphoryl lipid A (MPL, Ribi) isolated from endotoxins of Gram negative bacteria was shown to exert an adjuvant action on both the helper and suppressor branches of the immune response. Thus, toxicity is not a requirement for the adjuvant action of bacterial endotoxins.
2. MPL restored antibody production in aging mice and in the endotoxin low responding mouse strains C3H/HeJ and C57B110/ScN. In addition, MPL induced suppression in the C3H/HeJ strain.

GENERAL

1. Poly A·poly U, MDP and LPS increased phagocytosis in macrophages from young mice, but appeared to suppress this activity in aging virgin mice.
2. Aging breeder mice on the other hand were activated to increased phagocytosis similar to young mice, suggesting hormonal factors may control certain reactivities to adjuvants.

Characterization of Immune Suppression

Induced by Polyribonucleotides

by

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SUMMARY

Polyribonucleotide complexes, which have been shown to be potent adjuvants to the helper/inducer cells of the immune response of animals and humans were tested for their capacity to activate also cells involved in suppressing antibody synthesis. Poly A·poly U and poly I·poly C were found to inhibit murine antibody forming cells when given 1-6 days before antigenic stimulus. Attempts to identify the cell population responsible for the suppression revealed it could be transferred on day +2 after antigen with preparations enriched for both T or B cells as well as adherent cells. Thus, multiple cells may each contribute to the total suppression or a single cell type may be contaminating each of the isolated populations. Since the natural killer (NK) cell population has been shown by others to suppress the immune response, this likely candidate was renderred non-functional with anti-asialo antiserum with, however, no diminution in suppressive activity. Further experiments are required to define this adjuvant induced non-specific suppressor cell. Activation of suppression by the polynucleotides did not diminish murine resistance to challenge with either *Streptococcus pneumoniae* or *Pseudomonas aeruginosa*.

The induction of splenic suppressor cells by the polyribonucleotide complexes was found to be preceded by the appearance of a suppressor factor(s) in the serum 90 minutes after injection of adjuvant. This factor was heat stable, had a molecular weight greater than 30,000 Daltons and could be induced in sera from athymic nude mice.

MATERIALS AND METHODS

Polyribonucleotides. Poly A·poly U (The Midland Certified Reagent Company, Midland, TX) and poly I·poly C (Calbiochem-Behring, San Diego, CA) were prepared by annealing equal volumes of poly A and poly U each at 6 mg/ml

and poly I and poly C each at 3 mg/ml for 30 min. at 38°C, after which dilution was made to 300 µg/ml with phosphate buffered saline (PBS), pH 7.2. Intraperitoneal (ip) injections of 0.5 ml were used (150 µg/mouse) with control mice given 0.5 ml of PBS ip.

Mice. Balb/c mice, originally purchased from the Charles River Laboratory, Wilmington, MA were bred in our animal facilities by brother-sister matings. Mice of either sex were used at 7-12 weeks of age.

In Vivo Injections. PBS and the polynucleotides were injected ip in 0.5 ml. The antibody specific for NK cells, anti-asialo GM-1 (Wako Chemicals USA, Inc., Dallas, TX) was diluted 1:8 with PBS and 0.2 ml injected iv. Control mice were given 0.2 ml PBS iv.

In Vitro Cell Culture. Mice were sacrificed using ether, spleens sterilely removed and single cell suspensions prepared in Hanks balanced salt solution (HBSS). The cell debris was allowed to settle for 4 min. on ice after which the supernatant centrifuged at 1200 rpm for 10 min. If cell separations were to be carried out the red blood cells were lysed by adding 10 ml of 0.83% NH_4Cl-Tris buffer (0.17 M) for 5 min. and centrifuged as above. The cells were washed three times with HBSS and diluted to 1×10^7 cells/ml. If no cell separations were carried out the cells were resuspended in HBSS, centrifuged, resuspended in Click's medium (1) containing 10% fetal bovine serum, and diluted to 1×10^7 cells/ml with Click's medium. Sheep red blood cells (Wilfer Laboratories, Stillwater, MN) were used as antigen, after being washed 3 times in HBSS, counted and diluted to 4×10^8 cells/ml with Click's Medium. Duplicate cultures were set up for each experimental group by adding 1 ml of spleen cell suspension (1×10^7 cells) and 0.025 ml of SRBC (1×10^7 cells) to each culture dish. Cells were cultured at 5% CO_2, at 37°C and on day 4 were removed from the culture plates with a Pasteur pipet after scraping

gently with a rubber policeman, pooling the cells from duplicate wells. Each well was rinsed once with PBS and the tubes centrifuged for 10 min. at 1200 rpm. After centrifugation the supernatant was decanted, the cells resuspended in 5 ml PBS and added to the plates for PFC assay.

Separation of Adherent and Nonadherent Cells Using Baby Hamster Kidney (BHK)-Microexudate Coated Flasks. Approximately 2.5 x 10^8 spleen cells in 10 ml of HBSS and 5% FBS were plated on 250 ml flasks from which BHK monolayers had been removed with 10mM EDTA in 0.15 M PBS, pH 7.2. The cells were allowed to adhere 2 hours at 37°C in 5% CO_2 after which the nonadherent cells were removed by decanting and washing with HBSS. The adherent cells were recovered by adding 3 ml of 10mM EDTA in PBS and 7 ml of HBSS to the flask, incubating at 37°C for 5-10 min., decanting and rinsing the flask. Adherent cells were washed 2X to remove residual EDTA.

Separation of Adherent and Nonadherent Cells by Adherence to Plastic. Spleen cells were diluted to 1 x 10^7 cells/ml in Click's Medium and 1 ml of cells added to each culture dish. Cells were allowed to adhere for 2 hours at 5% CO_2, 37°C after which the nonadherent cells were collected and added to another culture dish for 30 minutes to remove any residual adherent cells. The adherent cells coating the first plate were washed with HBSS, covered with Click's medium and incubated until the nonadherent populations were ready. PBS-treated nonadherent cells from 1 x 10^7 starting spleen cells were added to both PBS-treated adherent cells from 1 to 10^7 starting cells and poly A·poly U-treated adherent cells from 1 x 10^7 starting cells. Likewise, poly A·poly U-treated nonadherent cells from 1 x 10^7 starting cells were added to both PBS-treated and poly A·poly U-treated adherent cells. Antigen was added and cells cultured to day 4 as described above.

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Separation of B and T cells by "Panning". The procedure originally described by Mage et al. (2) and later by Wysocki et al. (3) was used to adsorb a desired specific antibody to 100 x 15 mm petri dishes. Briefly, both the specific antibody (rabbit anti-mouse immunoglobulin or anti-mouse T cell serum, Cedarlane Laboratories Limited, Hornby, Ontario, Canada) and the filler antibody (normal rabbit immunoglobulin, DAKO, Accurate Chemical & Scientific Corp., Hicksville, NY) were diluted to 5 µg/ml in 0.05 M Tris buffer, pH 9.5 (Sigma Chemical Co., St. Louis, MO) after which the specific antibody was diluted 1:5 or 1:10 with the filler antibody to give a final specific antibody concentration of 0.5-1 µg 1 ml. Ten ml of the above mixture were placed on each sterile petri dish, swirled until the plate surface was covered and incubated on a level surface at room temperature for 40 minutes. The antibody mixture was decanted and the plates washed 4X by carefully pouring a small amount of PBS down the side of the dish and swirling, then decanting. A fifth wash was done with PBS and 1% FBS which was allowed to cover the dish until cells were ready to be added.

Following removal of adherent cells by attachment to BHK-microexudate coated flasks the nonadherent cells were diluted to 6.7×10^6 cells/ml in PBS and 5% FBS. Three ml of this cell suspension was then placed in each prepared petri dish and the plates incubated on a level surface at 4°C for 40 min. The plates were swirled and incubated an additional 30 minutes after which the unattached cells were decanted and the plates rinsed 4 times with PBS and 1% FBS. The bound cells were recovered by flushing the plate with 7-10 ml of PBS and 1% FBS. The recovered cell populations were centrifuged at 1200 rpm for 10 min., resuspended, counted and diluted in Click's medium for addition to cell cultures.

Hemolytic plaque assay. Antibody production was measured by utilizing a modified hemolytic plaque assay (4). Briefly, spleens were removed from mice sacrificed using ether on day 4, and single cell suspensions were prepared as described in "In Vitro Culture." After the first centrifugation the cells were washed once in HBSS and resuspended in PBS to a final concentration of 5 x 10^5 cells/ml. 60 x 15mm Nunc plastic culture dishes were coated with 2 mls of poly-L-lysine (50 µg/ml). After 15 min. the plates were washed with PBS, pH 7.2 and 2 mls of washed SRBC (diluted 1:20) were added. After 15 minutes the plates were swirled, allowed to settle for another 15 minutes, rinsed with PBS and covered with 1.5 ml of PBS. For in vivo experiments, 2.5 x 10^5 spleen cells were added and for in vitro experiments, 0.5 ml of the cell suspension was added to each plate and incubated in the presence of complement at 37C for 1 hour, after which plaques were counted.

Assay for Natural Killer (NK) Cell Activity. The NK assay was carried out as described by Murphy et al. (5). Briefly, 1 x 10^7 Yac-1 target cells in log phase growth were labeled for 1 hour with 200 µCi ^{51}Cr (Amersham Corp., Arlington Heights, IL) at 37°C, 5% CO_2. After labeling the cells were washed 3X, resuspended in 5 ml of RPMI 1640 (Gibco, Grand Island, NY) supplemented with Hepes buffer, 2-Mercaptoethanol, Penicillin/Streptomycin and 10% FBS. A viability cell count was done and the Yac-1 cells diluted to 2 x 10^5 viable cells/ml.

One day prior to the NK assay mice were injected ip. with PBS as control or with 150 µg of the polynucleotide complex. Approximately 24 hours later the mice were sacrificed and spleen cell suspensions prepared as in "culturing spleen cells in vitro."

After the first centrifugation the cells were resuspended in 2 ml RPMI and the red blood cells removed by adding 6 ml sterile water for 30 seconds

followed immediately by 2 ml of 3.5% NaCl. The resulting cell suspensions were centrifuged as above, resuspended in RPMI, counted and diluted to 2 x 10^7; 1 x 10^7 and 5 x 10^6 cells/ml for effector:target ratios of 200:1, 100:1 and 50:1 respectively. 0.1 ml of the above cell suspensions and 0.1 ml of the diluted ^{51}Cr-labeled Yac-1 cells were added to triplicate wells of a 96 well round bottom microtiter plate, which was then incubated for 4 hours at 37°C, 5% CO_2. Following incubation the plate was centrifuged at 800 rpm for 10 min. and a 0.1 ml aliquot from each well were counted for 1 minute in a Beckman Model 5500 gamma counter. Spontaneous release was determined by mixing 0.1 ml media with 0.1 ml Yac-1 cells and maximum release by mixing 0.1 ml 2N HCl with 0.1 ml Yac-1 cells. Percent cytotoxicity was calculated for each set of triplicates by the following equation:

$$\% \text{ cytotoxicity} = \frac{\text{cpm exptl.} - \text{cpm media}}{\text{cpm max.} - \text{cpm media}} \times 10$$

Statistical Analysis. Data were analyzed by a BMDP statistical program for a one sample "t" test after taking the logarithm_{10} of the ratio of each pair of groups compared.

RESULTS

In Vivo Antibody Suppression. Non-specific suppression of the in vivo antibody response by injection of the polyribonucleotide complexes one day prior to SRBC is demonstrated in Figure 1 and confirms our previous observations (6). A 50% suppression was exerted by poly A·poly U while poly I·poly C treatment induced a 65% suppression of antibody production.

Duration of Suppression. To determine the duration of the suppressive effect induced by the polynucleotide complexes, poly A·poly U and poly I·poly C were injected 10 (I·C only), 8, 6, and 4 days prior to antigen injected on day 0. PFC's were assayed 4 days later. Figure 2 summarizes the data showing suppression was still evident 6 days after poly I·poly C injection. By days 8

and 10 however, the suppressive effect had waned and was replaced by enhanced levels of antibody forming cells. Suppression induced by poly A·poly U lasted a shorter period of time, about 4 days under identical conditions.

In Vivo Transfer of Antibody Suppression. To determine if the suppression induced in vivo was transferrable by spleen cells, donor mice were injected with PBS as control or with the polyribonucleotide complex, sacrificed one day later and 1×10^7 spleen cells transfered iv to recipient syngeniec mice on either day 0 with antigen or day 2 after antigen. The hemolytic plaque assay was carried out on day 4. The results, (Figure 3) revealed that transfer on day 0 of cells from mice receiving either poly A·poly U or poly I·poly C complexes did not suppress antibody in recipient animals while transfer on day 2 gave statistically significant suppression. Variation was seen in individual experiments with pronounced suppression occurring on day $^+2$ in some experiments which was minimal in others.

In Vitro Suppression of Antibody. To facilitate determination of the cell(s) responsible for mediating the suppression, an in vitro system for suppression of antibody production also was studied. As is seen in Figure 4, when mice were injected with polyribonucleotide complexes ip one day prior to sacrifice and their spleens cultured in vitro with antigen, 40% suppression of PFC was exhibited by poly A·poly U and 65% suppression by poly I·poly C.

Antibody Suppression by Separated Poly A·Poly U Treated Spleen Cell Populations. To determine whether adherent and/or non-adherent cells were responsible for the suppression, splenic cells from polynucleotide injected mice were separated by adherence to plastic or BHK coated flasks and tested for their capacity to inhibit PFC formation in vitro. The results are shown in Fig. 5 and indicate greater suppressive activity using either procedure when both adherent and non-adherent polyribonucleotide treated fractions were

recombined. However, non-adherent cells alone from poly A·poly U treated mice when mixed with adherent cells from PBS-treated mice also exhibited a suppressive effect. On the other hand, in these experiments poly A·poly U treated adherent cells when mixed with PBS treated nonadherent cells did not result in statistically significant suppression of antibody production.

Further separation of non-adherent cells into B and T cell populations following addition to BHK microexudate coated flasks was effected by panning with rabbit anti-mouse immunoglobulin. The separated populations were then tested for their suppressive capacity. The average result from four such experiments is depicted in Fig. 6. Recombination of poly A·poly U treated (B + T + adherent) cells as the positive control gave statistically significant suppression of antibody. In addition, poly A·poly U treated (T + adherent) cells cultured with PBS treated B cells exerted significant suppression. The supression observed previously in Figure 5 when poly A·poly U treated adherent cells were cultured with PBS treated (B + T) cells which, however, was not statistically significant, now became significant due to less experimental variation in these further experiments. On the other hand, B cells, removed 1 day after injection of poly A·poly U when combined with PBS treated (T + adherent) cells significantly enhanced antibody production in vitro. All other poly A·poly U treated cell populations mixed with the complementary PBS treated populations resulted in antibody forming cells not significantly different from control.

When the same non-adherent cell populations as above were panned with anti-mouse T cell serum rather than rabbit anti-mouse immunoglobulin it was revealed that cells other than the T cell may be important in mediating suppression. In two experiments statistically significant suppression occurred in the groups containing poly A·poly U treated B or (B + adherent)

cells, suggesting that the active cell in mediating suppression actually may be a contaminating nonadherent cell not panned out with either rabbit anti-mouse immunoglobulin or anti-T cell serum (Figure 7).

NK Cell Involvement. Since the natural killer (NK) cell (a nonadherent cell) has been linked to immune-regulation (7), and the polynucleotides activate NK cells (8), several experiments were designed to investigate whether this cell was involved in polynucleotide induced suppression. Initially, both poly A·poly U and poly I·poly C were shown to activate NK cells when given at the same dose that induced suppression of antibody production (Figure 8). To determine if removal of NK cell activity with anti-sialo GM-1 antibody would affect polyribonucleotide-exposed spleens in their capacity to induce suppression, this antibody was injected iv 1 day prior to ip injection of PBS or polyribonucleotide complexes. As may be seen in Figure 9, this treatment was effective as NK cell activity was no longer evident in the poly A·poly U or poly I·poly C treated mice treated with anti-asialo antibody. However, suppression of anti-SRBC PFC formation by spleen cells from polyribonucleotide injected mice was retained despite injection of anti-asialo GM-1. Thus, under these conditions, no evidence could be attained that NK cells were mediating the suppression (Figure 10).

Microbial Challenge of Mice Following Poly A·Poly U Suppressed Antibody synthesis. The finding that suppression of antibody by poly A·poly U occurred over an approximate 4 day period raised the question as to whether this might be associated with a diminution in immunity to microbial challenge. Accordingly, mice were injected with 10 and 100 μg poly A·poly U and challenged 24 hr later with either *Pseudomonas aeruginosa* or *Streptococcus pneumoniae*. Paradoxically, a non-specific increase in reistance against both

organisms was induced by this adjuvant (Table 1). A similar phenomenon has been shown to occur with MDP (9) as well as LPS (10).

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LEGENDS FOR FIGURES

Figure 1. 150 µg of PBS or polyribonucleotide were administered on day -1. 1×10^8 SRBC given on day 0. Mice sacrificed and hemolytic plaque assay carried out on day 4. Data expressed are as mean of 8 or 9 experiments ± S.E.M. $^*p < 0.05$.

Figure 2. 150 µg of PBS or polynucleotide were administered to 3 mice each on day -10, -8, -6, or -4 with 1×10^8 SRBC given on day 0. Data expressed are as mean of 3 experiments ± S.E.M. (AU day -8 and IC day -10, 2 experiments). $^*p < 0.05$.

Figure 3. On day 0 or day 2 3 mice each were administered 1×10^7 spleen cells iv from syngeneic mice injected 24 hours earlier with PBS, 150 µg poly A·poly U or poly I·poly C. All mice were given 1×10^8 SRBC ip as antigen on day 0 with the hemolytic plaque assay carried out on day 4. Data are expressed as the mean of 4 to 7 experiments ± S.E.M. $^*p < 0.05$.

Figure 4. 150 µg of PBS or polynucleotide were injected into 1-2 mice on day -1. On day 0 mice were sacrificed and spleen cells placed in culture with antigen to day 4 when the hemolytic plaque assay was carried out. Data are expressed as mean of 8 or 9 experiments ± S.E.M. $^*p < 0.05$.

Figure 5. 150 µg of PBS or polynucleotide were administered to 2 to 6 mice each on day -1. The mice were sacrificed on day 0 and spleens pooled from similarly treated mice for use in cell separations. Following cell separations, cells were cultured in vitro to day 4 when the hemolytic plaque assay was done. Data are expressed as mean of 4 or 5 experiments ± S.E.M. $^*p < 0.05$.

Figure 6. 150 µg of PBS or polynucleotide were administered to 5 or 6 mice each on day -1. The mice were sacrificed on day 0 and spleens pooled from similarly treated mice for use in cell separation. Following cell separation by panning the component cell populations were added back together in the following approximate percentages: B cells-40, T cells-55, Adherent cells-5. Cells were cultured to day 4 when the hemolytic plaque assay was carried out. Data are expressed as mean of 4 experiments ± S.E.M. $^*p < 0.05$.

Figure 7. 150 µg of PBS or polynucleotide were administered to 5 or 6 mice each on day -1. The mice were sacrificed on day 0 and spleens pooled from similarly treated mice for use in cell separation. Following cell separation by panning the component cell populations were added back together in the following approximate percentages: B cells-60, T cells-35, Adherent cells-5. Cells were cultured to day 4 when the hemolytic plaque assay was carried out. Data are expressed as mean of 2 experiments ± S.E.M. $^*p < 0.05$.

Figure 8. Duplicate mice were injected on day -1 with 150 μg of PBS or polynucleotide. On day 0 the mice were sacrificed and assayed individually for Natural Killer cell activity against YAC-1 target cells at an effector: target ratio of 100:1. Data are expressed as mean of 5 experiments ± S.E.M. $^{*}p < 0.01$.

Figure 9. On day -2, three mice received anti-asialo GM1 iv or a control iv injection of PBS and on day -1 one mouse of each set of three was administered PBS, one mouse 150 μg of poly A·poly U and one mouse of 150 μg of poly I·poly C. The mice were sacrificed and the NK assay carried out on day 0 using an effector:target ratio of 100:1. Data are expressed as mean of 2 experiments ± S.E.M. $^{*}p < 0.05$.

Figure 10. On day -2, three mice received anti-asialo GM1 iv or a control iv injection of PBS and on day -1 one mouse of each set of three was administered PBS, one mouse poly A·poly U and one mouse poly I·poly C, all ip. All mice were given 1×10^8 SRBC ip as antigen on day 0 and sacrificed on day 4 for assessment of anti-SRBC antibody production. Data are expressed as mean ± S.E.M. of 3 experiments. ++Two experiments only. $^{*}p < 0.05$, $^{**}p < 0.001$.

FIGURE 1

SUPPRESSION OF IN VIVO ANTIBODY PRODUCTION BY PRE-INJECTION WITH POLYRIBONUCLEOTIDE

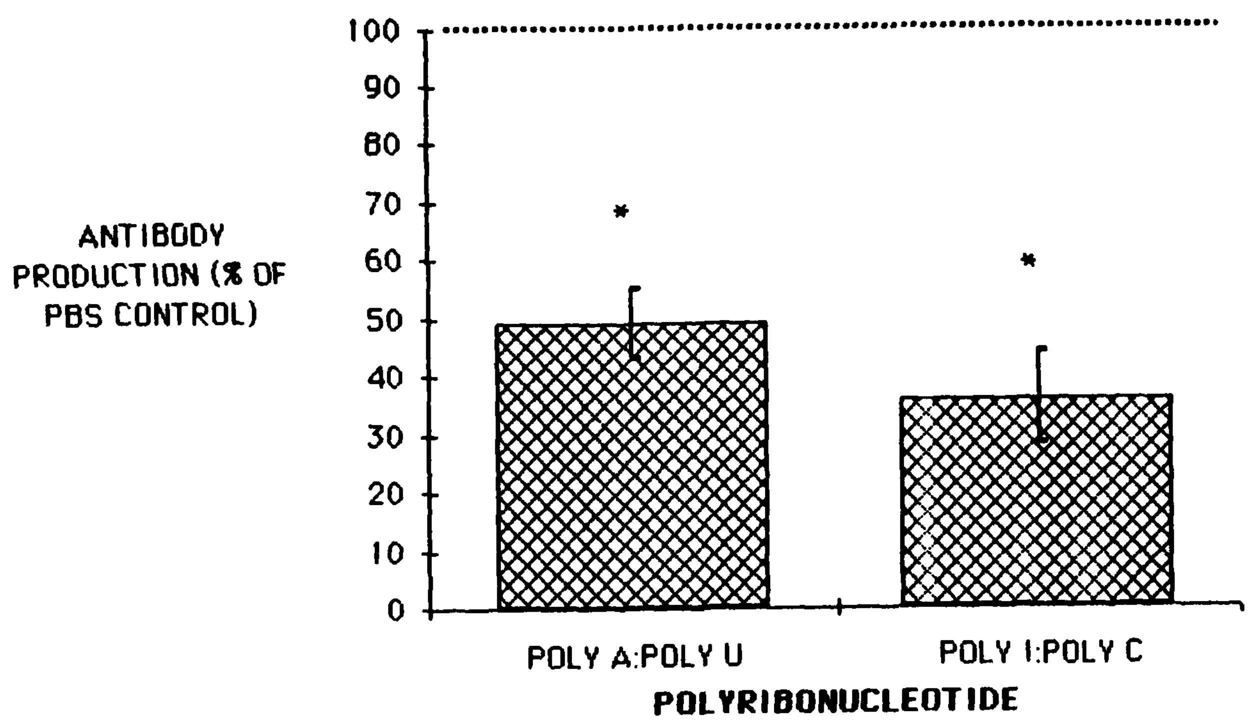

FIGURE 2

DETERMINATION OF THE DURATION OF THE SUPPRESSIVE EFFECT INDUCED BY THE POLYNUCLEOTIDES POLY A:POLY U AND POLY I:POLY C

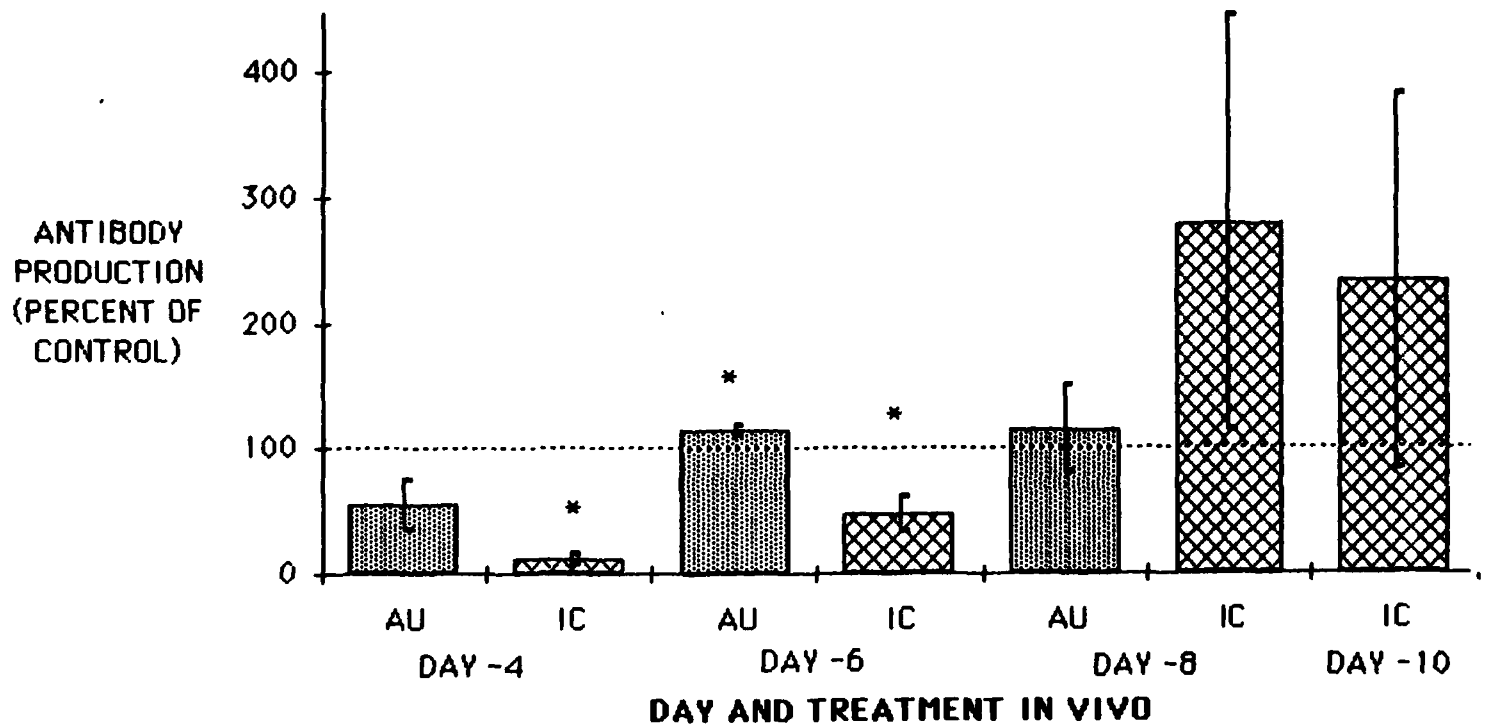

FIGURE 3

EFFECT OF TRANSFER OF POLYRIBONUCLEOTIDE-INDUCED SUPPRESSOR CELLS ON ANTIBODY PRODUCTION

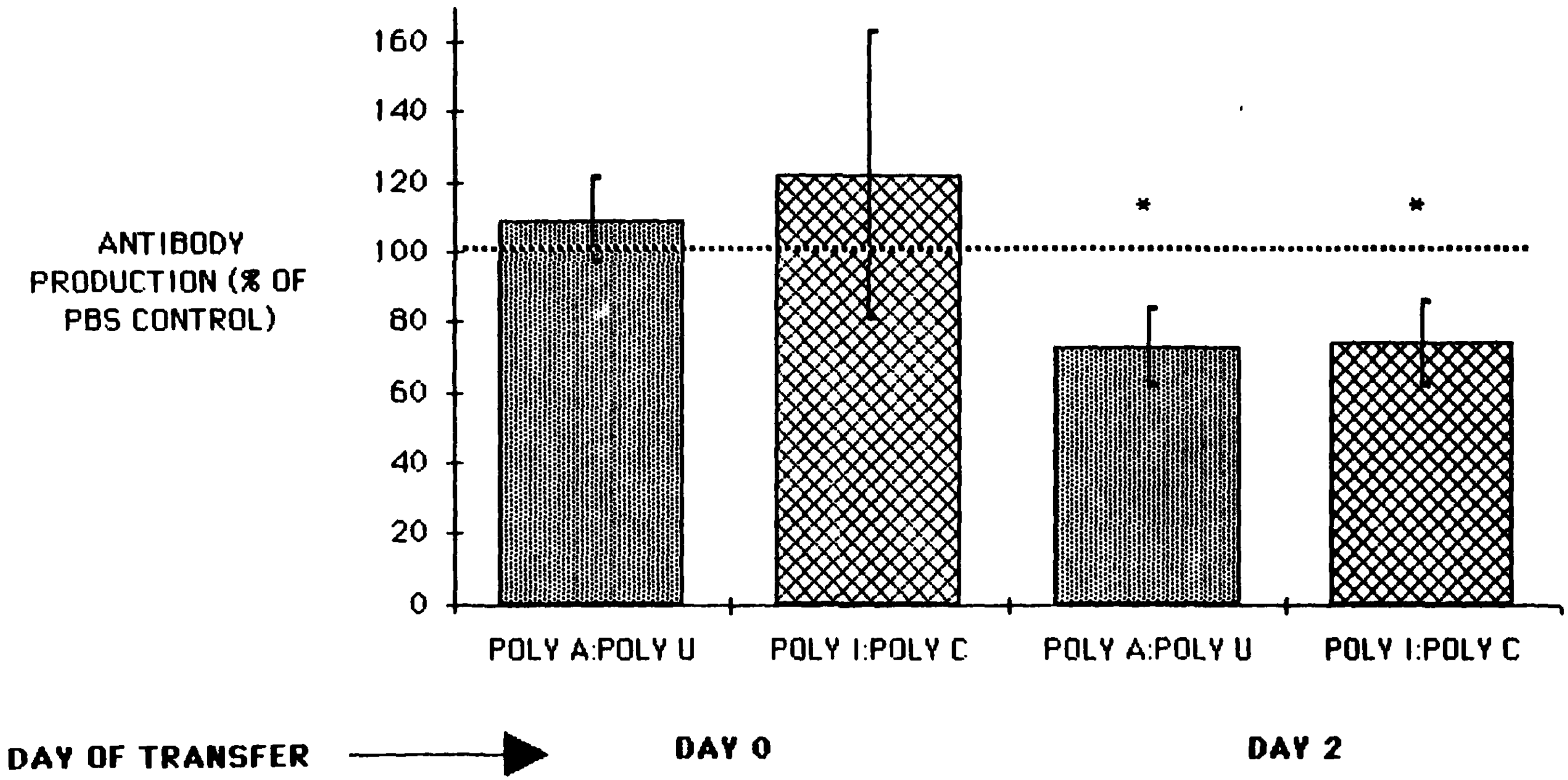

FIGURE 4

EFFECT OF POLYRIBONUCLEOTIDE PRE-INJECTION ON ANTIBODY PRODUCTION OF CELLS CULTURED IN VITRO ON DAY 0

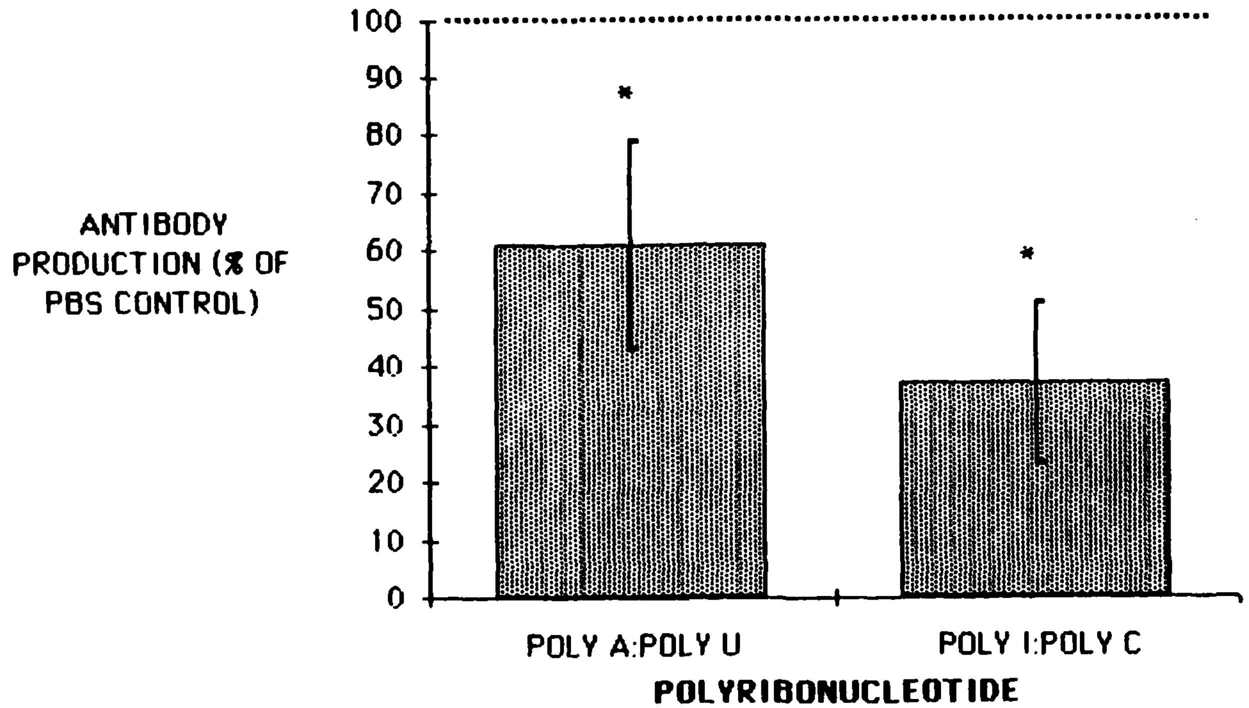

FIGURE 5

SUPPRESSION OF ANTIBODY PRODUCTION BY POLY A:POLY U-TREATED ADHERENT AND NONADHERENT CELLS

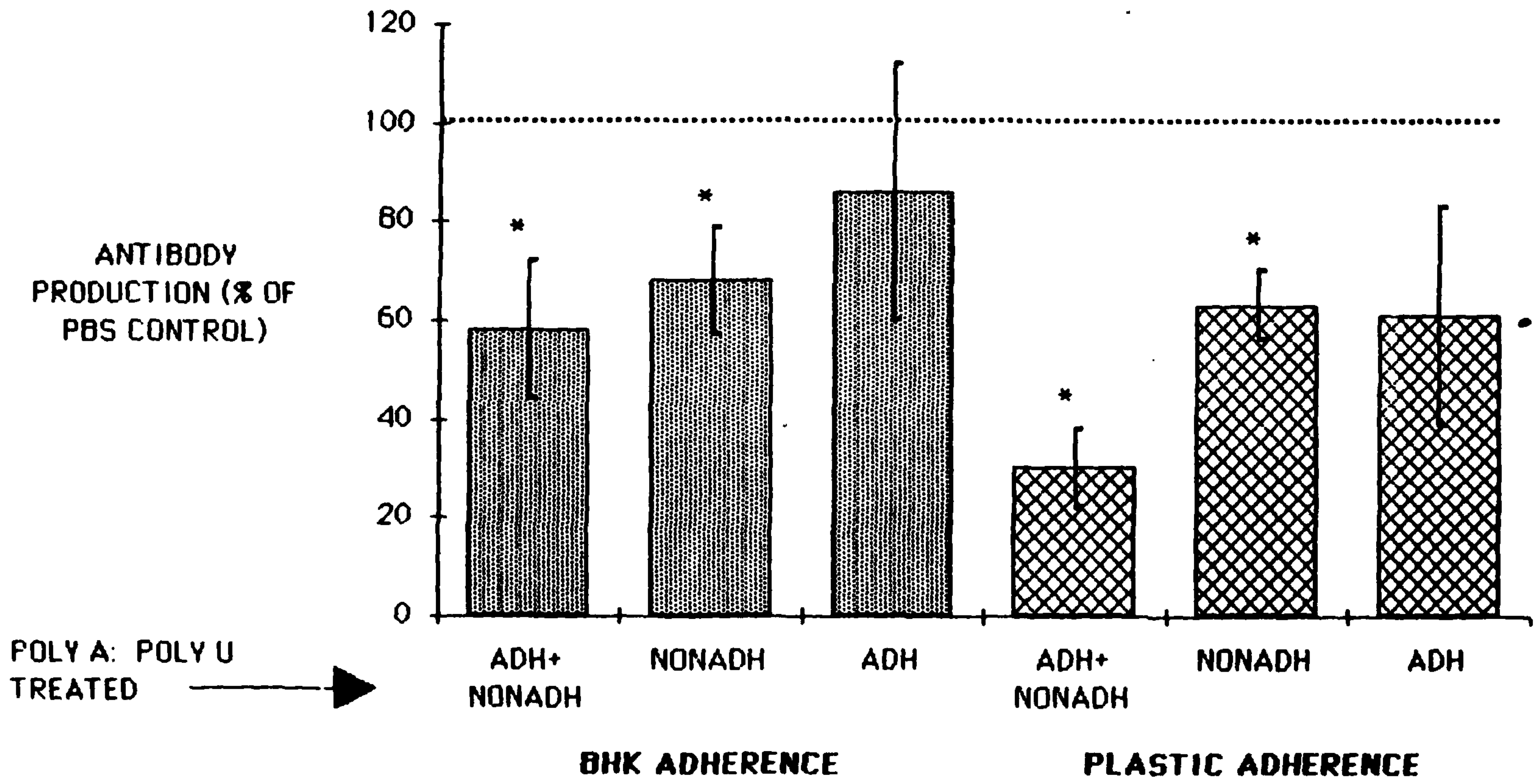

FIGURE 6

EFFECT OF POLY A:POLY U-TREATED B, T AND ADHERENT CELLS ON ANTIBODY PRODUCTION

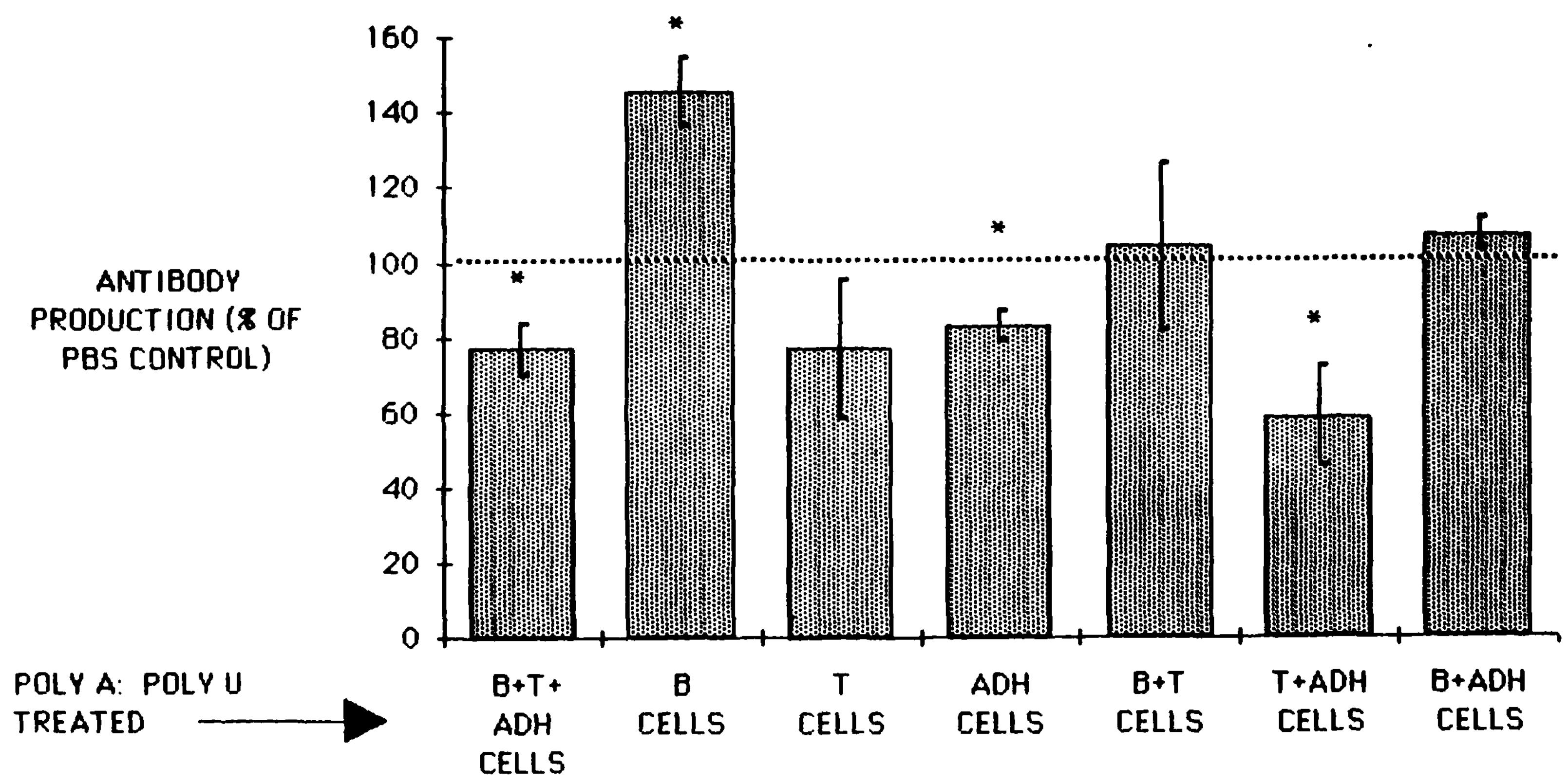

FIGURE 7

EFFECT OF POLY A:POLY U-TREATED B, T AND ADHERENT CELLS ON ANTIBODY PRODUCTION

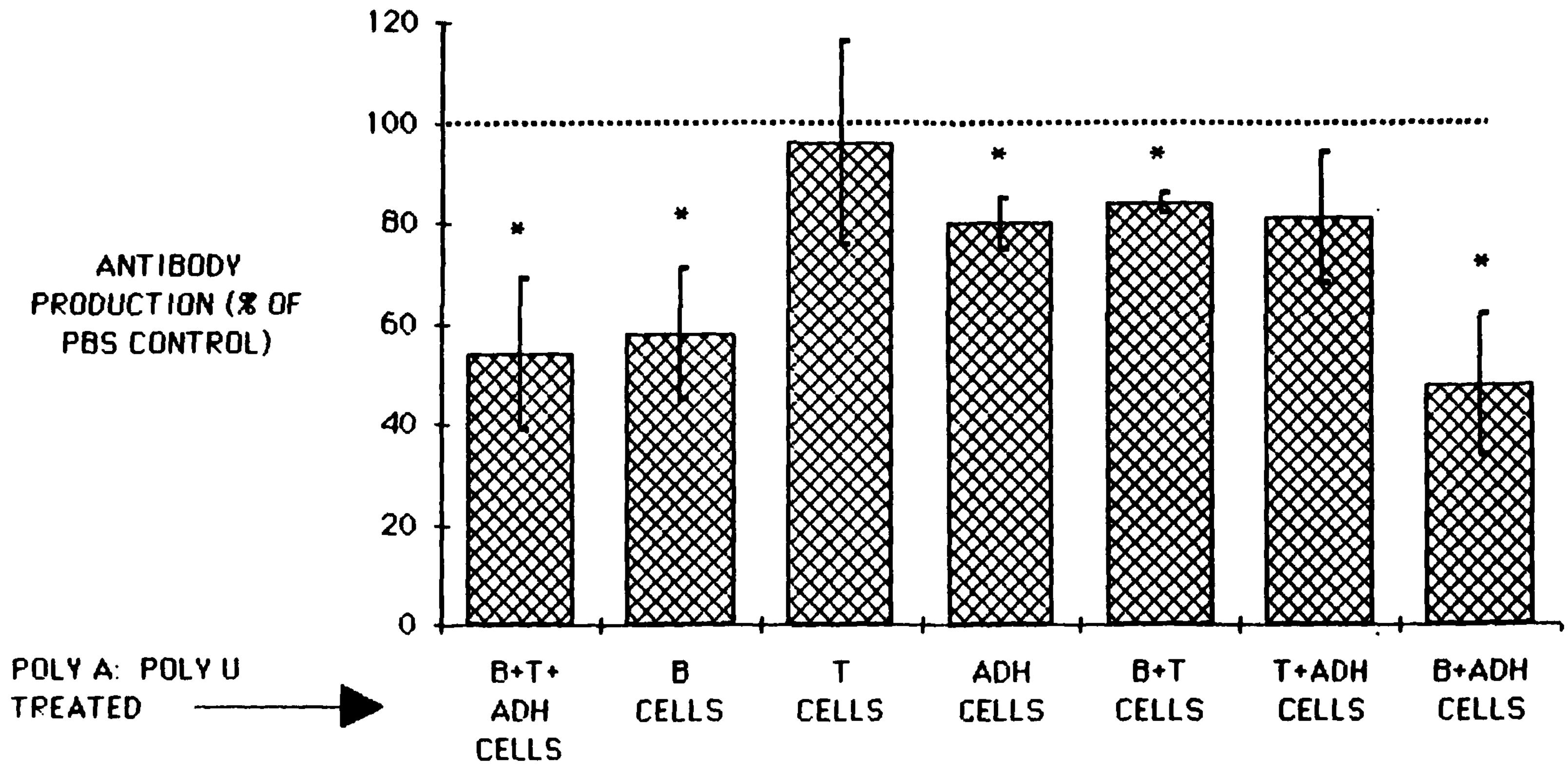

FIGURE 8

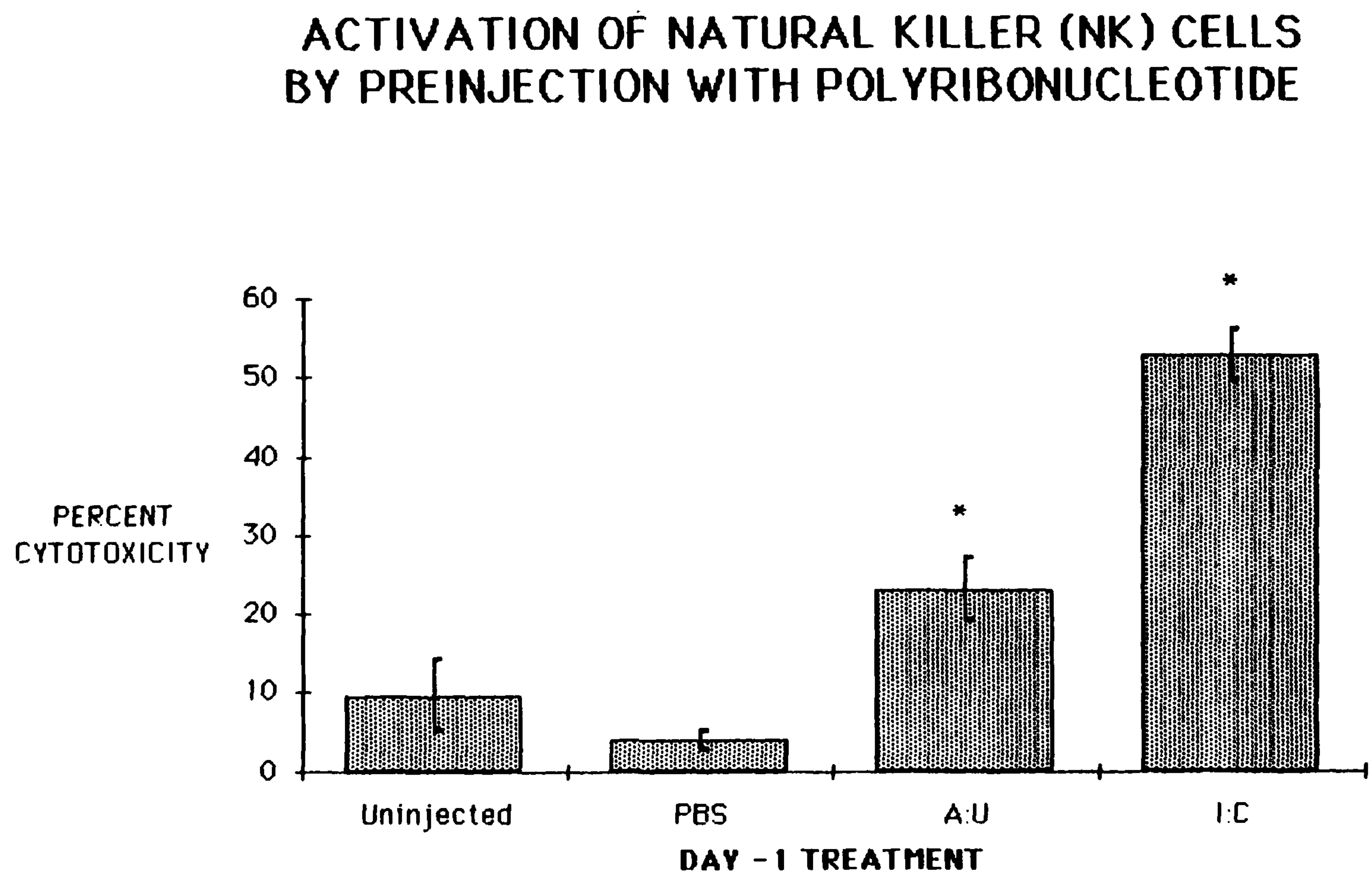
ACTIVATION OF NATURAL KILLER (NK) CELLS
BY PREINJECTION WITH POLYRIBONUCLEOTIDE
PERCENT
CYTOTOXICITY
60
50
40
30
20
10
0
*
*
Uninjected
PBS
A:U
I:C
DAY -1 TREATMENT

FIGURE 9

EFFECT OF PREINJECTION WITH ANTI-ASIALO GM1 ANTIBODY ON THE ABILITY OF POLYRIBONUCLEOTIDES TO ACTIVATE NATURAL KILLER (NK) CELLS

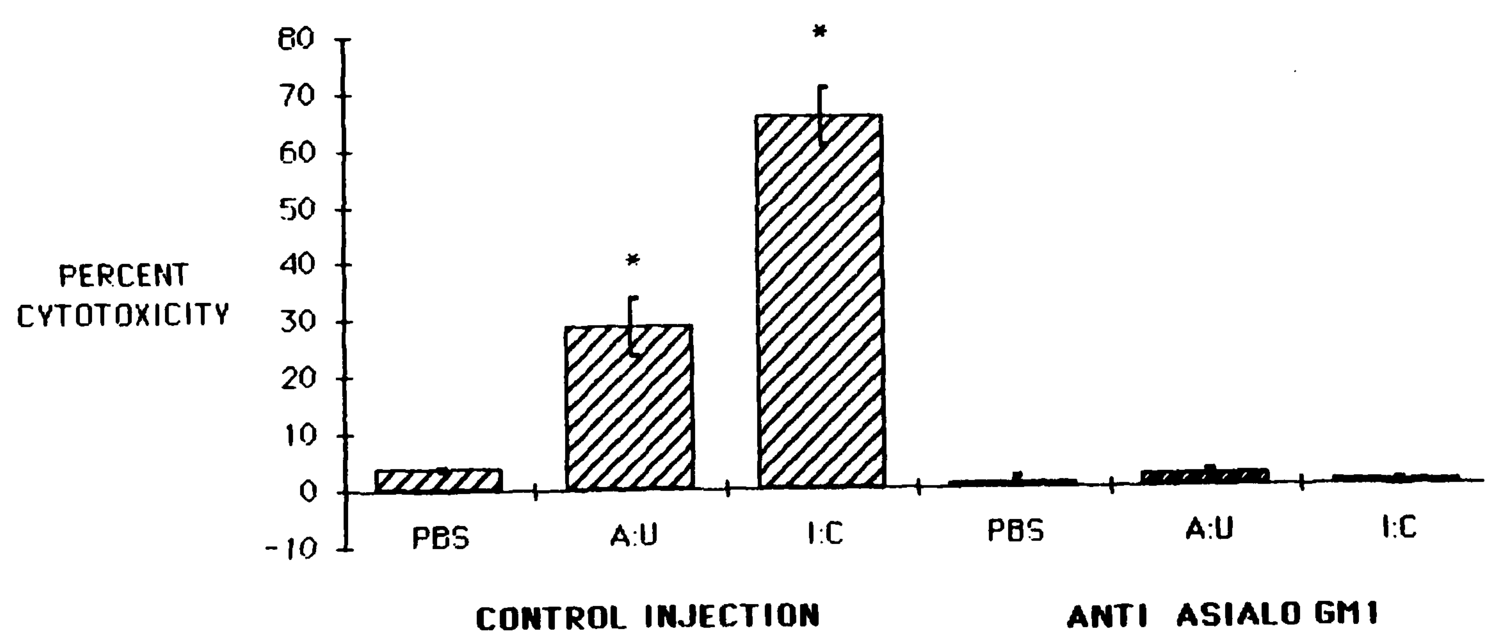

FIGURE 10

EFFECT OF PREINJECTION WITH ANTI-ASIALO GM1 ANTIBODY ON THE ABILITY OF POLYRIBONUCLEOTIDES TO INDUCE SUPPRESSION OF THE ANTIBODY RESPONSE

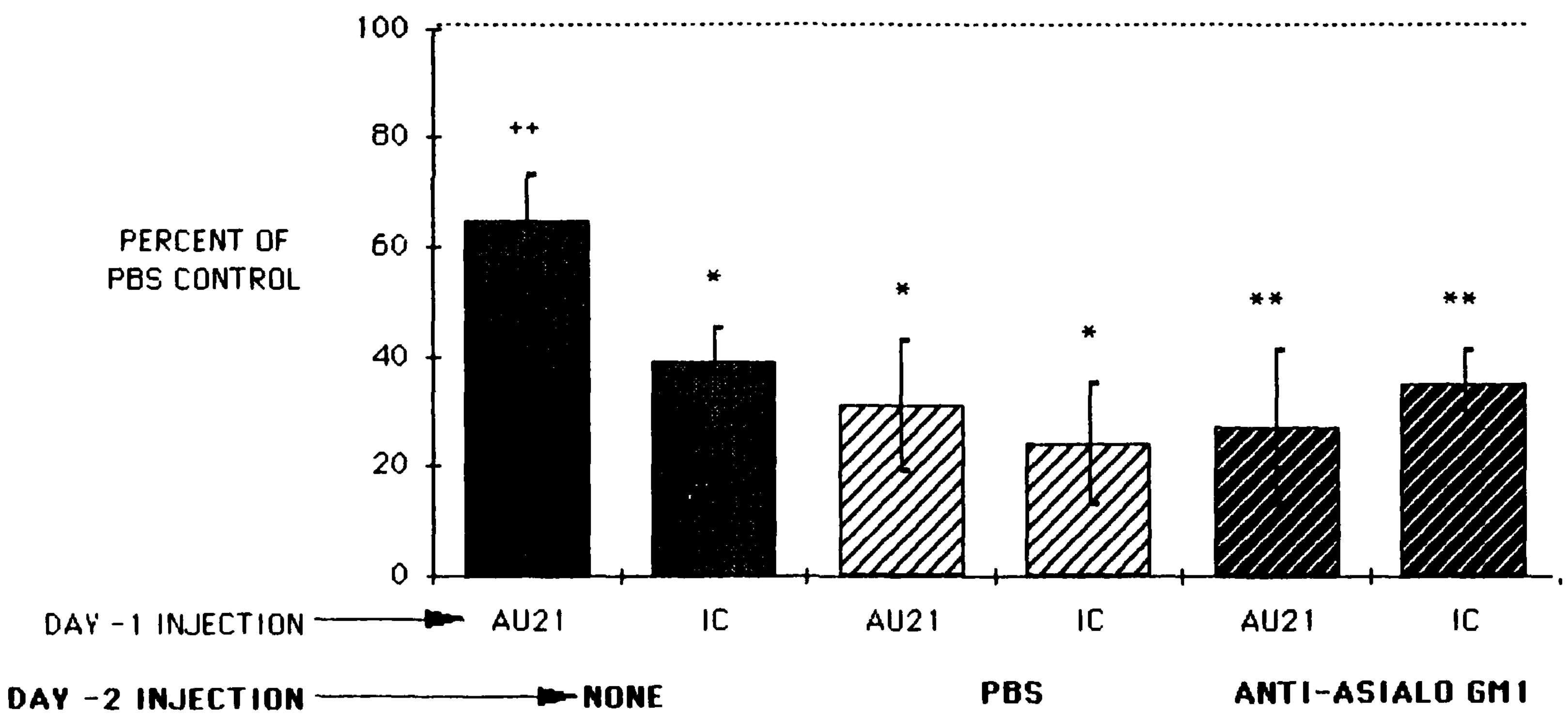

Table 1

Protection by Poly A·Poly U Against Infection

Ages of Mice	Challenge Organism	Adjuvant Injected*	No. Survivors** / No. Injected	% Survival
7 days	Pseudomonas aeruginosa (2×10^5, SC)	None Poly A·Poly U (10γ, SC) Poly A·Poly U (100γ, SC)	2/31 3/12 12/30	7 25 40
4 months	Streptococcus pneumoniae (2×10^2, iv)	None LPS, 10γ iv Poly A·U, 100γ iv	1/8 8/8 7/8	13 100 88

*Injected one day before challenge.

**Counted on day 15.

END

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